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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> A61K 39/385, 39/108, 39/02, 39/00, 39/38, 38/00, C07K 2/00, 4/00, 5/00, 7/00, 14/00, 16/00, 17/00, A01N 37/18	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/38171</b> <b>(43) International Publication Date:</b> 5 December 1996 (05.12.96)
<b>(21) International Application Number:</b> PCT/US96/08730 <b>(22) International Filing Date:</b> 3 June 1996 (03.06.96)  <b>(30) Priority Data:</b> 08/460,617                      2 June 1995 (02.06.95)                      US  <b>(71) Applicant:</b> DEPARTMENT OF THE ARMY, US GOVERN- MENT [US/US]; Moran, John, Office of Command Judge Advocate, HQ USAMRDC, Dept. of the Army, Fort Det- rick, Frederick, MD 21702-501 (US).  <b>(72) Inventors:</b> CASSELS, Frederick; 6317 Woodcrest Drive, Ellicott City, MD 21043 (US). ANDERSON, Jeffrey; 2015 Schuster Road, Jarrettsville, MD 21084 (US). CARTER, John, Mark; 35 Chicory Lane, Trenton, NJ 08638 (US).  <b>(74) Agent:</b> HENDRICKS, Glenna; P.O. Box 2509, Fairfax, VA 22031-2509 (US).		<b>(81) Designated States:</b> CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> METHODS OF RAISING ANTIBODIES AGAINST E.COLI OF THE FAMILY CS4-CFA/I  <b>(57) Abstract</b>  A consensus peptide of 36 amino acids has been designed which acts as an immunogen raising antibodies against the proteins of all members of the <i>E.coli</i> family CS4-CFA/I. While the N-terminus of members of this family of organisms shows a high degree of identity, the remainder of the sequence of the proteins shows much less homology across the strains. The region of the protein represented in the subunit encompasses known linear B- and T-cell epitopes of CFA/I. The consensus peptide has a high level of homology to strains bearing six different colonization factors. The consensus peptide is of the formula: VEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA. An alternative peptide, identified as consensus peptide 2 is of the formula: VEKNITVTASVDPTIDLLQADGSALPASVALTYSPA.		

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## APPLICATION FOR UNITED STATES PATENT

**Title:      Methods of Raising Antibodies Against E. Coli of the  
                 Family CS4-CFA/I**

5      **Field of the Invention:**

         This invention provides means of immunization of humans  
with a peptide or denatured protein against enterotoxigenic E.  
coli (ETEC) strains of the CS4-CFA/I family. The antibodies  
raised by the peptides and vaccines may be used as diagnostic  
10      agents to identify antigens of CS4-CFA/I bacteria.

**Background of the Invention:**

         The effect of E. coli in mammals is dependent on the  
particular strain of organism. Many beneficial E. coli are  
present in the intestines. Since the initial association with  
15      diarrheal illness, five categories of diarrheagenic E. coli  
have been identified and are presently recognized: enterotoxi-  
genic (ETEC), enteropathogenic (EPEC), enterohemorrhagic  
(EHEC), enteroaggregative (EAggEC), and enteroinvasive (EIEC).  
These categories are grouped according to characteristic  
20      virulence properties, such as elaboration of toxins and  
colonization factors and/or by specific types of interactions  
with intestinal epithelial cells. ETEC are the most common of  
the diarrheagenic E. coli and pose the greatest risk to  
travelers. E. coli of the family CS4-CFA/I are some of the  
25      more common enterotoxigenic E. coli. There is need for  
vaccines which are specific against this class of E. coli that  
give rise to antibodies that cross-react with and cross-protect  
against the more common members of the CS4-CFA/I family. There  
are six members of this family of ETEC fimbrial proteins,  
30      CFA/I, CS1, CS2, CS4, CS17 and PCF 0166. ETEC are responsible  
for high infant mortality in developing countries, with an  
estimate that almost 800,000 deaths per year due to these  
organisms. These organisms also cause illness in adult  
travelers to regions where the disease is endemic. No licensed  
35      vaccine exists against these organisms. The present vaccines  
being tested present problems related to manufacturing. So  
far, there has been no demonstration of significant efficacy  
of the prior art vaccines.

         Colonization factor antigens (CFA) of ETEC are important

in the initial step of colonization and adherence of the bacterium to intestinal epithelia. In epidemiological studies of adults and children with diarrhea, CFA/I is found in a large percentage of morbidity attributed to ETEC. The CFA/I is present on the surfaces of bacteria in the form of pili (fimbriae), which are rigid, 7 nm diameter protein fibers composed of repeating pilin subunits. The CFA/I antigens promote mannose-resistant attachment to human brush borders with an apparent sialic acid sensitivity. Hence, it has been postulated that a vaccine that establishes immunity against these proteins may prevent attachment to host tissues and subsequent disease.

Preferred vaccines are those that give consistent protection and that are relatively inexpensive to manufacture. Immunization with a single antigen, either a peptide or individual denatured protein, that could simultaneously protect against organisms bearing this family of differing colonization factors is needed. Hence, it is desirable to identify peptides that do not rely on living organisms for production. Experimental evidence indicates that when animals are immunized with intact fimbriae, cross-reactivity is minimal.

Testing for CFA/I, CS1, CS2, CS3, CS4, CS5 and CS6 has been the most common diagnostic evaluation attempted. CFA/I, CS3 and CS6 may occur alone, but with rare exception CS1 is only found with CS3, CS2 with CS3, CS4 with CS6 and CS5 with CS6. These colonization factor-bearing (CF) organisms have been reported to account for up to 75% or as little as 23% of the ETEC, depending on location of the study.

Detailed Description of the invention:

A study of proteins in E. coli belonging to the CS4-CFA/I family resulted in the finding that the N-terminal region of the protein maintains a high degree of sequence identity between members of this group. Immunological evidence shows that cross-reaction exists between members of the family CS4-CFA/I. Two problems of significant magnitude exist in the vaccine field that relate to immunization against varying strains of organisms: 1) is it possible to immunize against

several strains using a single antigen, whether a protein or a peptide, and 2) will antibodies raised against peptides or denatured proteins be effective against proteins having tertiary structures due to folding and disulfide bonding and to quaternary structures having not only tertiary structure, but also strong interaction between protein units? E. coli of the CS4-CFA/1 family have fimbriae on the surface of the cell which are composed of quaternary proteins composed of repeating protein units. Previously published experimental evidence indicates that when animals are immunized with intact fimbriae, cross-reactivity is minimal. However, when fimbriae are dissociated into subunits, i.e., the quaternary structure was broken down, response to linear epitopes occurs. The strain-specific antigens used in the examples were denatured protein subunits of the fimbriae.

A consensus peptide of 36 amino acids has been designed which raises acts as an immunogen raising antibodies against the proteins of all members of the E. coli family CS4-CFA/1. While the N-terminus of members of this family of organisms shows a high degree of identity, the remainder of the sequence of the proteins shows much less homology across the strains.

The region of the protein represented in the subunit encompasses known linear B- and T-cell epitopes of CFA/I. The consensus peptide has a high level of homology to strains bearing six different colonization factors. The consensus peptide is of the formula:

VEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA

An alternative peptide, identified as consensus peptide 2 of the formula:

VEKNITVTASVDPTIDLLQADGSALPASVALTYSPA

is made in the same manner as described in Example 1.

The consensus peptide was constructed based on the analogous peptides from CFA/I, CS1, CS2, CS4, CS17 and PCF 0166. The consensus peptide was shown to raise antibodies which agglutinated whole bacteria bearing colonization factors CFA/I, CS2, and CS1. The serum samples from animals that had been immunized with the denatured protein from strains 0166, CFA/I,

and CS2 were tested. Since the antibodies recognized the 0166 strain but did not cause agglutination of the organisms, it is clear that reactivity is not always evidenced by agglutination.

The analogous peptides of the denatured protein subunits from the various strains contain the following sequences:

5 CFA/1 VEKNITVTASVDPVIDLLQADGNALPSAVKLAYSPAS  
 CS1 VEKTISVTASVDPTVDLLQSDGSALPNSVALTYSPAV  
 CS4 VEKNITVTASVDPTIDILQADGSYLPTAVELTYSPAA  
 CS17 VEKNITVRASVDPKDLLQADGTSLPdSIALTYSA  
 10 PCF0166 VEKNITVTASVDPTIDILQANGSAL  
 CS2 AEKNITVTASVDPVIDLLQA

As indicated in the data provided herein, many of the denatured proteins gave rise to antibodies that are reactive with proteins of other strains as evidenced by precipitation studies on nitrocellulose and that are reactive with surface antigens of the fimbriae as evidence by agglutination of organisms.

Understanding of several of the sequences has been increased to encompass the following:

20 CFA/I

VEKNITVTASVDPVIDLLQADGNALPSAVKLAYSPASKTFESYRVMTQVHTNDATKKVIVK  
 LADTPQLTDVLNSTVQMPISVSWGGQVLSTTAKEFEAAALGYSASGVNGVSSSQELVISAA  
 PKTAGTAPTAGNYSGVSLVMTLGS

CS1

25 VEKTISVTASVDPTVDLLQSDGSALPNSVALTYSPAVNNFEAHTINTVVHTNDSKGVVVK  
 LSADPVLNSVLNPTLQIPVSVNFAGKPLSTTGITIQSNDLNFASSGVNKSSTQKLSIHADA  
 TRVTGGALTAGQYQGLVSIILTKST

CS4

VEKNITVTASVDPTIDILQADGSYLPTAVELTYSPAAsdFENYKIATKVHTNVINK  
 30 NVLVKLVNDPKLTNVLDStKQLkITVnwg ELNFGTSGVTGVSSNQD  
 lVIGATtnsQAPLAG

CS17

VEKNITVRASVDPKDLLQADGTSLPdSIALTYsxAxxx

PCF0166

35 VEKNITVTASVDPTIDILQANGSALPTAVDLTYLPGAKtFENYsVLxqiyt

CS2

AEKNITVTASVDPTIDLMQSDGTALPSAVNIAYLPGEKRF

Peptides or denatured proteins of the invention may be conjugated to other moieties such as other proteins, peptides, hydrophobic moieties and proteosomes. The peptides, whether free or conjugated, may be administered by mouth in protected form. The peptides may thereby be presented to the mucosa of the intestinal tract to raise mucosal immune response. For example, the peptides may be protected by encapsulation in accord with the teachings of U. S. Patent 5,417,986, which is incorporated herein by reference in its entirety. Peptides and denatured proteins may also be formulated in other protection means such as liposomes or microcrystals. The protected peptides or denatured proteins are particularly useful for administration by mouth.

Vaccine compositions may be introduced into the patient by conventional means, including parenteral routes (subcutaneous, intradermal, intramuscular) and by direct application to mucous membranes. Lyophilized compositions may be "snorted" into the nasal cavity. Dosage will depend on the particular agent administered. The method of administration directly to the mucous membranes is exemplified herein. It should be understood that the use of this technology disclosed herein has particularly wide applications for making of immunogens for administration to the mucous membranes. The immunogenic compositions containing the peptides may contain adjuvants known in the art, such as Freund's adjuvant or alum.

#### Materials and Methods

Conlonization factors (CF) from ETEC strains of each member of the CFA/I family were purified and run on polyacrylamide gels. Stained bands from transfers of the gels to PVDF membrane were excised and subjected to automated Edman degradation. Peptides were synthesized by solid phase automated Fmoc chemistry. The peptides were prepared by solid phase peptide synthesis using a 430A peptide synthesizer (Applied Biosystems, Inc., Foster City, CA) using 9-fluorenylmethoxycarbonyl (Fmoc) amino and t-butyl side chain protecting groups supported by HMP resin (p-hydroxymethylphenoxymethyl polystyrene resin). All solvents and side chain protected

amino acids were also obtained from Applied Biosystems and used in accord with the instruction provided therewith. Following synthesis, protecting groups were removed by acid hydrolysis using anisole and ethanedithiol as scavengers. Amino acid analysis and reverse-phase high pressure liquid chromatography were used to determine amino acid content and peptide purity respectively.

The consensus peptide was conjugated to bovine serum albumin (BSA) or tetanus toxoid. When the peptide was conjugated to as indicated below, a cysteine residue was added at the terminal end of the peptide to provide the following consensus peptide:

CVEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA

The albumin or toxoid was then iodoacetylated. The peptide was mixed with the acetylated albumin or toxoid. (Sulfide bonds are thereby formed between cysteine residues providing a conjugated protein.) BSA-dexran is also appropriate for conjugation with these antigens.

Immunogenic compositions contained complete Freund's adjuvant and were administered to rabbits subcutaneously on day 1. On day 21, a booster shot was given. The booster shot differed from the first dosing in that a smaller amount of protein was given and the complete Freund's adjuvant was replaced with incomplete Freund's adjuvant. On day 32 the animals were bled.

**Example 1:**

Rabbits were bled, then immunized on day 0 with a composition containing 280  $\mu$ g peptide/BSA conjugate in Freund's complete adjuvant. On day 21, the animals were boosted with 140  $\mu$ g peptide/BSA conjugate in Freund's incomplete adjuvant. Blood was drawn on day 32. The interaction of antibodies raised against the specific antigens of the denatured proteins of the various strains was studied by comparing interaction of serum from the animals obtained on day 0 with interaction of serum from the animals obtained on day 32 by Western blot. In all instances, the Western blot was negative for reaction with serum obtained on day 0. The Western blot data on interaction



of immune serum collected on day 32 with the denatured proteins is given below with 0 being no reaction and 4 being a strong reaction:

Titer	1:50	1:500	1:5000	1:50000
CS1	4	4	4	4
CS2	4	4	4	
CS4	4	4	3	2
CS17	4	3	2	0.5
0166	4	1	3	
CFA/I	4	3	2	

**Example 2:**

An immunogenic composition is prepared containing 280  $\mu\text{g/ml}$  of a conjugate of a peptide of the formula:

VEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA bound to BSA through a cysteine in complete Freund's reagent.

**Example 3:**

A immunogenic composition is prepared containing 400  $\mu\text{g/ml}$  of a peptide of the formula:

VEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA  
in complete Freund's adjuvant.

**Example 4:**

Rabbits were given a composition containing 400  $\mu\text{g}$  peptide of the formula:

VEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA in complete Freund's adjuvant. The response was evaluated as in Example 2:

Titer	1:50	1:500	1:5000	1:50000
CS1	4	4	2	1
CS2				
CS4	2	0	0	0
CS17	2	0	0	0
0166	4	2		
CFA/I				

**Example 5:**

The same study was done comparing antibodies raised to denatured proteins of 0166.

Titer	1:1000	1:10000	1:100000
CS1	3	0.5	0
CS2	2	1	0
CS4	2	0.5	0
CS17	3	0.5	0
0166	4	3	1
CFA/I	3	0.5	0

**Example 6:**

Effect of antibody raised to denatured protein was studied in the manner of example 5.

Titer	1:1000	1:10000	1:100000
CS1	4	3	0.5
CS2	4	2	0
CS4	3	1	0
CS17	3	1	0.5
0166	4	1	0
CFA/I	3	0	0

**Example 7:**

Studies were conducted to determine whether antibodies raised to the peptide would cause agglutination of whole bacteria of various strains. Antibody to three consensus peptide antigen preparations were compared: 1) peptide conjugated to bovine serum albumin (aPepBS), 2) free peptide (PepFr) and 3) peptide conjugated to tetanus toxoid (PepTT). The tetanus toxoid was conjugated to the peptide using the described above for conjugation to BSA. The three preparations were used to immunize two animals each. The serum was then contacted with whole bacteria and the slides were inspected for agglutination of the bacteria.

CF	aPepBSA	aPepFr	aPepTT
CS1	1/2	0/2	1/2
CS2	2/2	0/2	2/2
CS4	0/2	0/2	0/2
CS17	0/2	0/2	0/2
0166	0/2	0/2	0/2
CFA/1	1/2	0/2	2/2

In view of the test data, it is seen that the data indicates that consensus proteins can give rise to antibodies

that are reactive with denatured proteins of species of the family CF4-CFA/1 and that such antibodies also cause agglutination of organisms of more than one strain of E. coli of the CF4-CFA/1 family. However, it is also seen that conjugation to a larger molecule provides improved properties to the peptides for purposes of raising antibodies to the whole bacteria and the proteins of these organisms.

The preparation of analogous peptides of the various strains of this family of organisms containing the sequences disclosed above may also be used as vaccine cocktails. The peptides could be prepared by the methods disclosed herein or by other methods of peptide synthesis known in the art. The denatured proteins themselves may be used as antigens for preparation of vaccines in order to raise antibodies against E. coli of the family CFA/I. The analogous peptides or denatured proteins could be conjugated to proteins in accord with the methods taught herein. Hence, the instant invention also relates to vaccine compositions containing at least one denatured protein from an E. coli of the family CFA/I in a pharmaceutically acceptable carrier which may contain an adjuvant. The denatured fimbrial protein or any of the peptides disclosed herein may be conjugated to molecules which enhance antigenicity and administered as vaccines.

Additional peptides for use in accord with the teachings herein can also be designed based on the amino acid sequences disclosed herein for use in vaccines. A peptide of at least 30 residues would be preferred. The antigenicity of the smaller peptides would be enhanced by conjugation to larger molecules known in the art to increase antigenicity.

## CLAIMS:

1. A peptide containing the sequence:  
VEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA.
2. A peptide of claim 1 which is  
CVEKNITVTASVDPTIDLLQADGSALPSAVALTYSPA
3. A peptide of claim 1 conjugated to a molecule which increases antigenicity.
4. A peptide of claim 1 conjugated to albumin.
5. A composition of matter comprising a peptide of claim 1 in a pharmaceutically acceptable carrier.
6. A composition of claim 5 containing, additionally, an adjuvant.
7. A composition of matter comprising at least one denatured fimbrial protein from an E. coli of the family CS4-CFA/I in a pharmaceutically acceptable carrier.
8. A method of immunizing a mammal against disease caused by E. coli of the family CS4-CFA/I comprising the steps of:  
(1) denaturing fimbrial protein from at least one species of said E. coli,  
(2) administering an immunogenic effective amount of a denatured protein obtained in step 1 to a mammal susceptible to disease caused by E. coli of the family CS4-CFA/I.
9. A method of immunizing a mammal against disease caused by E. coli of the family CS4-CFA/I by administration of a composition of claim 5.
10. A composition of claim 5 wherein the consensus peptide is

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conjugated to a larger molecule.

11. A composition of claim 10 wherein the larger molecule to which the consensus peptide is conjugated is tetanus toxoid.

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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08730

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.  
US CL : 424/184.1, 241.1, 194.1, 234.1, 236.1, 193.1; 530/300, 324; 514/2, 12  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 241.1, 194.1, 234.1, 236.1, 193.1; 530/300, 324; 514/2, 12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

registry, genbank, hcaplus, pir, swissprot, ageneseq, wpids, biosis, aps  
search terms, fimbriae, pili, pilin, carrier, adjuvant, immuniz?, registry names, author/inventor name, e. coli, cs4/cfal, colonization factor antigen

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CARON et al. A plasmid-encoded regulatory gene, rns, required for expression of the CS1 and CS2 adhesins of enterotoxigenic Escherichia coli. Proc. Natl. Acad. Sci. USA. February 1989, Vol. 86, pages 963-967, see entire document.	1-11
Y	GREWAL et al. Induction of colonization factor antigen I (CFA/I) and coli surface antigen 4 (CS4) of enterotoxigenic Escherichia coli: relevance for vaccine production. Vaccine. 1993, Vol. 11, No. 2, pages 221-226, see entire document.	1-11

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08730

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HALL et al. Purification and analysis of colonization factor antigen I, coli surface antigen 1, and coli surface antigen 3 fimbriae from enterotoxigenic Escherichia coli. Journal of Bacteriology. November 1989, Vol. 171, No. 11, pages 6372-6374, see entire document.	1-11
A	WILLSHAW et al. Cloning of regulator genes controlling fimbrial production by enterotoxigenic Escherichia coli. FEMS Microbiology Letters. 1991, Vol. 82, pages 125-130, see entire document.	1-11
Y	CASSELS et al. Colonization factors of diarrheagenic E. coli and their intestinal receptors. Journal Industrial Microbiology. 1995, Vol. 15, pages 214-226, see entire document.	1-11
A	US 4,443,549 A (P. L. SADOWSKI) 17 April 1984, see entire document.	1-11
Y	US 4,971,794 A (M. A. LINGGOOD ET AL.) 20 November 1990, see entire document.	1-11
Y	SOMMERFELT et al. Genetic relationship of putative colonization factor O166 to colonization factor antigen I and coli surface antigen 4 of enterotoxigenic Escherichia coli. Infection and Immunity. September 1992, Vol. 60, No. 9, pages 3799-3806, see entire document.	1-11
Y	RON et al. Microbial Surface Components and Toxins in Relation to Pathogenesis. 1991, New York: Plenum Press, pages 37-53, see entire document.	3-11
X	KARJALAINEN et al. Molecular cloning and nucleotide sequence of the colonization factor antigen gene I of Escherichia coli. Infection and Immunity. April 1989, Vol. 57, No. 4, pages 1126-1130, see entire document.	1
X	CASSELS et al. Analysis of Escherichia coli colonization factor antigen I linear B-cell epitopes, as determined by primate	1, 4-6
Y	responses, following protein sequence verification. Infection and Immunity. June 1992, Vol. 60, No. 6, pages 2174-2181, see entire document.	2, 3, 7-11
A	McCONNELL et al. Antigenic homology within human enterotoxigenic Escherichia coli fimbrial colonization factor antigens: CFA/I, coli-surface-associated antigens (CS)1, CS2, CS4 and CS17. FEMS Microbiology Letters. 1989, Vol. 61, pages 105-108, see entire document.	1-11



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08730

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RUDIN et al. Monoclonal antibodies against enterotoxigenic Escherichia coli colonization factor antigen I (CFA/I) that cross-react immunologically with heterologous CFAs. Infection and Immunity. October 1994, Vol. 62, No. 10, pages 4339-4346, see entire document.	1-11
X	WO 92/01703 A1 (EMORY UNIVERSITY) 06 February 1992, see entire document.	1, 4-6
Y		2, 3, 7-11
Y	TACKET et al. Purification, morphology, and genetics of a new fimbrial putative colonization factor of enterotoxigenic Escherichia coli O159:H4. Infection and Immunity. May 1987, Vol. 55, No. 5, pages 1063-1069, see entire document.	1-11

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/08730

**A. CLASSIFICATION OF SUBJECT MATTER:**  
IPC (6):

A61K 39/385, 39/108, 39/02, 39/00, 39/38, 38/00; C07K 2/00, 4/00, 5/00, 7/00, 14/00, 16/00, 17/00; A01N 37/18